STERNBERG (G.M.)

Gen. R. Sturgis
compliments.

A contribution to the study
of the bacterial organisms ×

A CONTRIBUTION TO THE STUDY OF THE BACTERIAL ORGANISMS COMMONLY FOUND UPON EXPOSED MUCOUS SURFACES AND IN THE ALIMENTARY CANAL OF HEALTHY INDIVIDUALS. ILLUSTRATED BY PHOTO-MICROGRAPHS.[1]

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INTRODUCTION.

THE observations recorded in the following paper and the photo-micrographs by which it is illustrated, were made in the Biological Laboratory of Johns Hopkins University, Baltimore, Maryland, during the months of June, July and August, 1881, at which time the writer was acting under the orders of the National Board of Health, and was engaged in special investigations which occupied a considerable portion of his time, and to which this study was necessarily subsidiary.

Microscopists have long been familiar with the fact that a variety of bacteria are constantly found in the alimentary canal of healthy individuals, and that the examination with a sufficiently high power of saliva or fæces never fails to demonstrate the presence of a multitude of these micro-organisms of various forms. Some microscopists to whom this fact is familiar, and whose studies have shown them the widely extended distribution of the bacteria, both within and without the human body, have shown a disposition to ridicule the idea that these minute organisms, so universally present, are capable under any circumstances of playing so important a rôle in the etiology of infectious and epidemic diseases as has been ascribed to them by believers in the "germ-theory." It must be admitted that many extravagant and unfounded claims have been made by over-enthusiastic supporters of this theory, and that a scientific conservatism is very essential to him who would estimate at their true value the facts developed by the numerous re-

[1] Read at the Cincinnati meeting of the A. A. A. S., Aug. 18th, 1881.

searches which have been made relating to the bacteria. The literature of the subject is already enormous, and the yearly additions to it seem to grow almost in geometrical progression, showing the rapidly increasing interest in the subject among physicians, sanitarians and men of science generally, due to a more general appreciation of the importance of the questions involved.[1]

It is evident that the time has passed when the spirit of investigation can be arrested by the exhibition under the microscope of the bacteria found in the saliva or fæces of a healthy individual and the magisterial dictum of an "expert microscopist" that these minute organisms are entirely harmless.

That there are many widely distributed forms (species?) which are ordinarily harmless, can not be questioned, but that pathogenic bacteria exist, either as distinct species or as physiological varieties (Pasteur) of common forms, is now definitely proven.

No apology, then, is needed for a study of this nature, the object of which is to place upon record photographic representations of the common bacterial organisms found in the bodies of healthy individuals and some observations relating to their physiological properties and the best method of studying them.

It is evident that a precise knowledge of the morphology and development—life-history—of these common forms is an essential prerequisite to the recognition of unusual forms and to the enlightened study of the possible relation of such forms to any particular disease with which they may be found associated.

I call attention, however, *en passant*, to the fact that recent researches indicate that too much importance has heretofore been attached to morphological distinctions, and that not only may the same organism present distinct morphological peculiarities in different stages of its development, but that during the same stage differences in size, if not in form, may result from conditions relating to the environment—temperature, composition and reaction

[1] NOTE.—In the bibliography compiled by Magnin ("The Bacteria," Little, Brown & Co., Boston, 1880) and added to by myself, but which can by no means be considered complete, the references from 1830–40 are seven; from 1840–50, twelve; from 1850–60, seventeen; from 1860–70, sixty-three; from 1870–80, above three hundred and fifty. In the second volume of the "Index Catalogue to Library of the Surgeon-General's Office," just published, four closely printed pages are required for the references relating to "Charbon" alone.

of medium, presence or absence of oxygen, etc. On the other hand, organisms morphologically undistinguishable from each other may possess different physiological properties.

The researches of some of the pioneers in this field of investigation, and especially the discovery by Davaine of a bacillus in the blood of Anthrax and of Obermeier of a spirillum in that of relapsing fever, led many to anticipate that organisms morphologically distinct might eventually be discovered for each specific disease.

This expectation has not been realized, and the germ-theory has been vigorously attacked by conservative opponents who have properly pointed out the morphological identity of *Bacillus anthracis* and *B. subtilis*, and of *Spirochaete Obermeieri* and *S. plicatile* which is not infrequently found in the mouth of healthy individuals. This argument has, however, lost its force, and the common and usually harmless bacteria around us have acquired a new importance since it has been shown by Pasteur,[1] Buchner,[2] Greenfield,[3] Grawitz,[4] and others, that, by special methods of cultivation, pathogenic varieties may be developed from harmless organisms, and that, by certain treatment, deadly bacteria may so far lose their virulence as to produce only a mild, though protective form of disease. In a recent study[5] of "A Fatal Form of Septicæmia in the Rabbit produced by the Sub-Cutaneous Injection of Human Saliva" I have obtained experimental evidence pointing in the same direction.

A brief reference to these facts is all that I can permit myself in the present paper, but I desire to call attention to certain possibilities which remain after the negative demonstration has been made that no organisms are present in the blood of patients suffering from a certain disease—that is, none demonstrable with the highest powers of the microscope as at present perfected. This

[1] "De l'attenuation du virus du choléra des poules." C. R. Ac. des Sc., XCI, p. 373-80.

[2] "Ueber die experimentelle Erzeugung des Milzbrand-Contagiums aus den Heupilzen. München, 1880."

[3] "Further Investigations on Anthrax and Allied Diseases in Man and Animals." Brown Lectures, I–V; London Lancet, 1880, pp. 965–966; 1881, pp 3-4, 91-94, 163–164.

[4]

[5] See Bulletin National Board of Health, April 30, 1881, and succeeding article in the present number of this Journal.

negative demonstration by no means proves that the disease in question is not a germ disease, for the habitat of the parasite may be elsewhere than in the blood, which may not offer the proper conditions for its development and from which it may be excluded by vital or mechanical obstacles.

Bacteria are always present in the alimentary canal of healthy men and animals, but that they do not find their way into the blood-stream, or if so, are quickly disposed of, has been amply proven by the negative results of microscopical examinations and culture-experiments.

In the form of septicæmia in the rabbit which I have recently studied, *l. c.*, I have invariably found an abundance of micrococci in the effused serum in the sub-cutaneous cellular tissue of an animal recently dead, but these organisms are not always found in the blood, and my observations indicate that they only invade the circulating fluid during the last hours of life. Micro-organisms have been found in many other localities without their presence being revealed by a microscopical examination of the blood; *e. g.*, in effused liquids in the pleural and peritoneal cavities, in pyæmic abscesses, and in various tissues and organs of the body. I have quite recently found an abundance of minute bacilli in the substance of the heart of a rabbit, which died as the result of the sub-cutaneous injection of a contaminated water (unpublished experiment).

The possibility that pathogenic bacteria may become parasitic upon the bronchial mucous membrane, or in the air-cells of the lungs, should also be borne in mind. But when we consider the extent of the alimentary tract, the variety of substances taken as food and drink, and the ready access which micro-organisms have to this human culture-apparatus, kept as it is at a constant temperature and supplied with pabulum suited to their development, it seems probable that this is the locality where pathogenic organisms may most frequently find the conditions favorable to their multiplication. This view is supported by many facts connected with the epidemic prevalence of pestilential diseases, and it is generally admitted that patients suffering from typhoid fever and cholera may sow the seeds (germs?) of these diseases in the discharges from their bowels.

It is unnecessary to dwell further upon the possibilities in this direction which make it important that the bacterial organisms

present in the human body should be studied by modern scientific methods—photography, isolation and cultivation in various media, injection into animals, etc., etc., but I will refer for a moment to another possibility which has occurred to me, which should, I think, receive the attention of chemists and physiologists.

What is the rôle of these micro-organisms which are constantly present in the alimentary canal of men and animals?

The fact that they are parasites does not exclude the possibility of their playing an important physiological rôle in the animal economy.

I am not speaking of accidental or occasional parasites, but of those which have probably been the commensals of man, and of the inferior animals frequented by them, from the earliest times. It can hardly be possible that in the process of evolution the presence of these parasites has had no influence upon the host, or that, to go no further back, in the gradual change from the mode of life and habits of a nomadic savage to that of a civilized man, the change in environment has had no modifying influence upon these micro-organisms, which laboratory experiments show to be so susceptible to changes in temperature and in the composition of the medium in which they are placed.

The question is frequently asked, "If bacteria are such terrible things, how is it possible that we can exist upon the earth surrounded and infested as we are by them?" Certainly there would be an end to all animal life, or rather there would never have been a beginning, if living animals had no greater resisting power to the attacks of these parasites, which by numbers and rapid development make up for their minute size, than has dead animal matter.

On the other hand, but for the power of these little giants to pull to pieces dead animal matter, we should have dead bodies piled up on all sides of us in as perfect a state of preservation as canned lobster or pickled tongue, and there being no return to the soil of the materials composing these bodies, our sequoias and oaks would dwindle to lichens and mosses, and finally all vegetation would disappear and the surface of the earth would be a barren and desolate wilderness, covered only with the inanimate forms of successive generations of plants and animals.

Section 1.

A number of authors[1] have given more or less extended accounts of the micro-organisms found in the human mouth, and their accounts agree so well with each other and with the results of my own observations, that I should hardly think it necessary to record these, but for the fact that I am able to present photographic representations of the organisms described for comparison with the illustrations drawn by other observers.

The special advantages which I claim for this method of illustration are set forth in a paper contained in the last volume (1880) of the Transactions of the American Association for the Advancement of Science.

I would especially call attention to two recent papers, one by Butlin, of England, and the other by Rappin, of France, both of which are illustrated and show careful study.

[1] *Remak.* "Diagnostische und pathologische Untersuchungen." Berlin, 1845, s. 221.

Pfeufer. "Der Mundhöhlen-Katarrh." Henle u. Pfeufer. Ztchft. f. Rat. Med., Bd. 7, 1849, s. 180.

Miguel. "Untersuchungen über den Zungenbeleg." Prager Viertel-Jahrschft., 1850, Bd. 28, s. 44.

Robin. "Végétaux Parasites." Paris, 1853, p. 345.

Neidhardt. "Mittheilungen über die Veränderungen der Zunge in Krankheiten." Arch. der wissensch. Heilkunde, Bd. V, 1861, s. 294.

Hyde Salter. Todd's "Cyclopædia of Anatomy and Physiology." Art. "Tongue." Vol. IV, pt. 2, p. 1161.

Hallier. "Die pflanzlichen Parasiten." Leipzig, 1866.

Kolliker. "Handbuch der Gewebelehre." 5te Auflage, 1867, ss. 348–349.

Farlie Clarke. "Diseases of the Tongue." London, 1873, p. 93.

Billroth. "Coccobacteria septica." Berlin, 1874, s. 94.

Robin. "Leçons sur les Humeurs." Paris, 1874, p. 550.

Koch. "Untersuchungen über Bacteria." Cohn's Beiträge zur Pflanzen, Bd. II, Hft 3, s. 399.

Butlin. "On the Nature of the Fur on the Tongue." Proc. Royal Soc., London, Vol. XXVIII, p. 484.

Rappin. "Des Bactéries de la Bouche." Thése de Paris, No. 145, April, 1881.

Methods of Research.

Collecting.—I have found the following to be the most satisfactory method of collecting bacteria for examination with high powers and for photography.

The slightest possible smear of the material to be examined is allowed to dry upon a thin glass cover, and to secure a sufficiently uniform layer, it is usually best to spread it while moist with the end of a glass slide.

Material is obtained from the mouth by scraping the surface of the tongue, or of the teeth, with a clean instrument; from the female vagina by a speculum or digital examination; and from the mouth of the male urethra by applying a thin glass cover directly to the moist mucous membrane at the extremity of the canal.

Staining.—A five-cent bottle of aniline violet ink furnishes an ample supply of staining fluid of the best quality. Two or three drops of this placed upon the thin cover will very quickly—one to three minutes—give to the bacterial organisms attached to its surface a deep violet color. The cover is then to be washed by a gentle stream of pure water and is ready for immediate examination, or may be mounted for permanent preservation over a shallow cell containing a solution of potassium acetate (Koch's method), carbolic acid water (2–5 per cent.), camphor water, or simply distilled water.

Photographing. — To make satisfactory photographs of the smallest bacteria it is necessary to use a staining fluid which will give stronger photographic contrast, as the violet is transparent for the actinic rays. I have employed for this purpose aniline brown (recommended by Koch), or iodine solution (iodine 2–5 grains, potassium iodide q. s. to dissolve, distilled water 100 grains).

A recent writer (Soubbotine[1]) advises the use of osmic acid as a fixing solution to be used in advance of staining. This is doubtless desirable when specimens of blood or thin sections of tissue containing bacteria are to be examined, as the normal histological elements are better shown, but the method possesses no special advantages so far as the demonstration of vegetable organisms is concerned. It must be remembered that aniline solutions often

[1] Arch. de Phys., 2e série, VIII, p. 479.

contain a granular precipitate which might be mistaken by a novice for deeply stained micrococci.

I cannot here give a detailed account of the technique of the art of photo-micrography, but will simply say that there are many difficulties to be overcome, and that the best results can only be obtained by the use of first-class objectives of high power, and by skilful manipulation in the preparation of slides and projection of a well-defined image, supplemented by a sufficient knowledge of the technique of photography to ensure the making of well-timed, well-developed, and properly intensified negatives. For one who has not the services of a practical photographer at his command, the dry-plate process offers many advantages.

Culture-experiments.—A knowledge of the life-histories and physiological properties of the various vegetable parasites which infest the human body can only be obtained by well-devised and carefully conducted culture-experiments. This method of research is still in its infancy, but it has already given valuable results and must doubtless be our main reliance for the advancement of science in this direction. My own experiments have been made chiefly with a view to testing methods and are preliminary to more extended studies which I hope to make in the future.

Culture-cells in which a drop of fluid—aqueous humour, etc.—containing the organisms to be observed, is in contact with a thin glass cover and surrounded by a limited quantity of air, are useful and convenient for certain purposes, especially for the continuous study of successive stages in the development—life-history—of bacterial organisms. But the method of Pasteur—cultivation in gross in sterilized fluids contained in glass flasks—offers decided advantages so far as the isolation, preservation, and cultivation of special forms, and the exclusion of atmospheric germs is concerned; and, also, because the considerable quantity of fluid used gives material for physiological experiments—injections into animals, etc.

The method which I have found most satisfactory, after a considerable number of experiments with various forms of apparatus, is a modification of that of Pasteur which I will proceed to describe in detail.

The culture-flasks which I employ are shown in Figure 1, Plate XI, supported in small bottles in the position in which they are introduced into the culture-oven.

The larger one, in the centre, is made from a Florence flask, the neck of which has been drawn out into a capillary tube in the flame of a Bunsen burner. The smaller flasks are of my own manufacture, and are made from glass tubing of about ¼ inch diameter. Bellows operated by the foot and a flame of considerable size—gas or alcohol—will be required by one who proposes to construct these little flasks for himself, but they could doubtless be obtained at small expense from any thermometer-maker. A little practice has enabled me to turn out twenty or thirty in an hour, and I have found it much easier to make new tubes than to clean old ones. I therefore throw them away when they have been once used.

After blowing the bulb the lower end is drawn out in a capillary tube and hermetically sealed in the flame. In this condition the flask which is already sterilized by heat, may of course be preserved indefinitely, free from contamination by atmospheric germs.

To introduce a liquid into the flask, heat the bulb slightly, break off the sealed extremity of the tube and plunge it beneath the surface of the liquid. If the liquid has already been sterilized, temperary exposure to the air while several of the little flasks are being filled is not likely to result in the introduction of atmospheric germs—for any organisms which fall upon the surface of the liquid will be arrested there for a time, unless they are submerged by mechanical means—stirring.

I have found it best, however, not to trust to the sterilization of my culture-liquid previously to its introduction into the flasks, and am in the habit of filling a considerable number of them at one time with filtered chicken-*bouillon*, Cohn's fluid, hay-infusion, or whatever culture-fluid I may desire to use; and, after again hermetically sealing the capillary extremity of the tubes, sterilization of the contents is effected by heat.

This is accomplished by placing the flasks in a bath of oil, melted parafine or concentrated salt-solution, and maintaining them at a temperature of about 105° C. for an hour or more. Occasionally a flask which has an exceptionally thin bulb will explode, and care must be taken by the operator that the hot oil is not thrown into his face by such an accident. This possibility makes it desirable that a bath should be used having a fixed boiling-point not exceeding 105°, and which consequently does not

require watching. I have found a concentrated salt-solution to fulfil this requirement.

After sterilizing, the flasks are washed to remove the salt-solution from their surface. They are then placed in a culture-oven kept at a temperature of 95–100° Fah. (36–38° C.) for three or four days to test the success of the previous operation—sterilization.

If the liquid contents remain transparent and no mycoderma has formed upon the surface during this time, the flasks may be put aside for future use and can be preserved indefinitely.

The process of sterilization sometimes causes a floculent precipitate to form when albuminous fluids are employed, although they may have been previously boiled and filtered. This might lead to the suspicion that they had broken down, but for the fact that this precipitate is already present when the flasks are introduced into the culture-oven, and no subsequent change takes place.

To inoculate the liquid contained in one of these flasks with organisms from any source, the extremity of the tube is broken off with forceps, the bulb being dependent, and by the application of gentle heat—the heat of the hand is usually sufficient—enough air is forced out to cause a little fluid to be drawn into the tube upon immersing its extremity in the liquid and allowing the air in the bulb to again contract by cooling.

A little experience will enable the operator to inoculate one tube from another, to introduce a minute quantity of blood containing organisms directly from the veins of a living animal, etc., without any danger of contamination by atmospheric germs. No other method with which I am acquainted offers such security as to sterilization of the culture-fluid and exclusion of foreign germs; and a somewhat extended experience in a recent experimental study, "A Fatal Form of Septicæmia," etc., *l. c.*, has convinced me that it has also decided advantages on the score of convenience.

The bottle which supports the inverted flask protects the capillary extremity from dust, and labels are conveniently attached to it. The formation of a mycoderma upon the surface of the fluid is readily recognized, and contained organisms soon settle to the bottom of the tube. Small quantities of fluid are conveniently obtained for microscopical examination by breaking off the end of the tube, forcing out a little of the contents on a clean slide and immediately sealing the extremity again in the flame of a lamp.

Another form of apparatus which I have found very useful is that of Lister, a slight modification of which is shown in Figure 2, Plate XI.

In the apparatus as described by Lister a conical wine-glass contains the culture-liquid, and this is covered by a circular glass plate; the whole being protected from dust by a bell-jar which rests upon a ground-glass plate.

When proper precautions are taken, a sterilized liquid may be preserved in this apparatus for any length of time without undergoing perceptible change. I have used a bell-shaped glass cup having a stem drawn out and sealed in the flame of a Bunsen burner, in preference to a wine-glass, as it is more easily sterilized by heat without danger of breakage. This is supported by a bottle as shown in the figure, and I have commonly dispensed with the use of a glass cover, the use of which is directed by Lister, as I have not found this to be essential to the success of my experiments.

Section II.

Description of Plates, Remarks upon Morphology, etc.

The most conspicuous vegetable organism found in the healthy human mouth, and the one which will usually first attract attention upon microscopical examination with low powers, is the well known *Leptothrix buccalis*, Robin. This I have never failed to find, in greater or less abundance, in material scraped from the surface of the tongue, or in accumulations dislodged from between the teeth. Often it is found in tufts and masses which indicate a vigorous growth, and again it may only occur in the form of short rods sparsely intermingled with the normal histological elements of the saliva, as shown in Figure 2, Plate XIII. But in this case it is probable that a careful search would reveal the presence in the mouth of the microscopic plantations and garden-beds from which these fragments were detached.

As might be expected, those who make frequent use of the tooth-brush leave less soil upon the surface and in the interstices of the teeth for the growth of this and other vegetable parasites. No amount of care, however, will keep the mouth entirely free from them, and the observations of Butlin (*l. c.*) show that the fur upon the tongue, which is rarely entirely absent even in healthy

individuals, is in great part made up of this and other vegetable parasites.[1]

In Figure 1, Plate XII, several filaments of *Leptothrix* are shown in which evidence of breaking up into joints is seen; and in Figure 2 of the same plate we have a mass of jointed filaments that seem rather to come under the definition of *Bacillus* than of *Leptothrix*, as given by Magnin in accordance with the classification of Cohn. This author says: "The *Leptothrix* differ from the Bacilli by their filaments being very long, adherent, very slender, and indistinctly articulated."

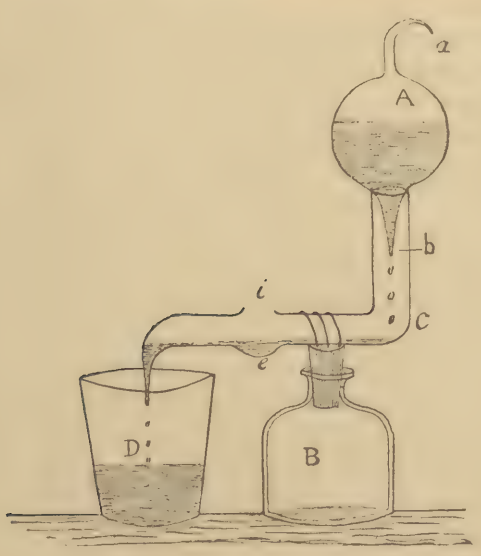
These characters seem to me to be very uncertain and unsatisfactory, inasmuch as *Bacillus subtilis* and *B. anthracis*, in one stage of their development, are *very long and slender and indistinctly articulated;* and, on the other hand, we have here a Leptothrix broken up into very distinct joints not distinguishable from those of Bacillus.

The filaments represented in Figure 1 are from a specimen of saliva obtained directly from my own mouth, while those in Figure 2 were developed in a culture-apparatus of special construction (see below) in which a constantly renewed supply of pabulum—chicken-*bouillon*—was passed through a small chamber, freely supplied with air, containing saliva scraped from the surface of my tongue. Butlin did not succeed in his efforts to cultivate this organism. He says: "I made many attempts to separate them in order to produce this fungus in a purer form by cultivation, but did not succeed in doing so. Although this fungus did not develop under artificial conditions in the presence of micrococcus and other fungi, it is highly probable that its development takes place freely upon the surface of the tongue."

It seems probable that my success in the experiment above mentioned is to be attributed to the constantly renewed supply of pabulum and the free access of oxygen, conditions which are certainly present in the mouth, where the surfaces upon which this parasite grows are constantly bathed with saliva and supplied with air. The author above quoted is of the opinion that the organism in question is identical with *Bacillus subtilis*, and in certain cases he observed "highly refractive spherical bodies which appeared to be spores" in some of the filaments. I have also observed short

[1] Butlin found "on 68 healthy tongues—fur on all except one. On 178 tongues of persons suffering from disease or accident—fur on all except two."

rods containing a single spore at one extremity in specimens of my own saliva examined in New Orleans during the summer of 1880. But at this time similar rods with spores were abundant in certain culture-fluids in my laboratory, and I supposed these to be *Bacilli* accidentally present in my mouth and differing from the common *Leptothrix buccalis.* This is a question, however, which can only be determined by culture-experiments, and I would suggest that the best way to settle it would be to cultivate the *Leptothrix* in an artificial saliva constituted as nearly as possible like normal saliva—but, of course, without the histological elements—and in a culture-apparatus such as was used in my single experiment above referred to. This apparatus is made as follows: A glass receiver *A* having two capillary tubes, one *a* to admit air, and one *b* to permit the gradual escape of the contained culture-fluid, is supported by the bent tube *C*, which is maintained in an upright position by being tied to the cork of a bottle *B*, which answers as a support for the apparatus. Mercury may be placed in this bottle to give it steadiness. The bent tube *C* has a reservoir *e*, which is freely exposed to the air by means of the opening *i*. The organism to be cultivated is introduced into this reservoir. The overflow from *e* is received in the beaker *D*. No attempt is made to exclude atmospheric germs, as the object of the apparatus is to supply, as nearly as possible, the identical conditions found in the human mouth.



My observations have not been sufficiently extended to justify me in an attempt to describe all of the organisms which are occasionally found in the human mouth, and I shall only refer briefly to the fact that the recorded observations of microscopists indicate that nearly every common bacterial organism known is sometimes found in this situation. This is no more than we should expect, as the germs of these various organisms are widely distributed in the atmosphere and must be deposited upon the moist mucous membrane during inspiration. Their development here will of course depend upon whether the conditions are favorable or otherwise. As these conditions vary within certain limits, we naturally find at different times and in different individuals a variety of organisms present in the buccal secretions differing from those common forms which observations made at distant points[1] show to be constantly present under normal conditions.

Among the varying conditions found in the mouths of individuals considered healthy may be mentioned, a greater or less abundant flow of saliva, a difference in the reaction of this fluid, the presence of decayed teeth, various habits as to food, drink, use of tobacco, etc. The variety of odors to be detected in the breath is sufficient to show that conditions may vary, and it may be that a sufficiently thorough research would result in the establishment of a causal relationship between the presence of certain organisms and the peculiar and offensive odors referred to.

When engaged in the microscopical examination of foul gutter-water and in culture-experiments with various putrifying organic substances, in New Orleans, La., during the autumn of 1880, I not infrequently found nearly every organism in my own mouth which was present in the putrifying liquids under examination, including *Bacterium termo*, *Bacillus subtilis*, *Spirillum undula*, and a variety of minute spherical and rod-like forms difficult to classify except under the general heading of micrococci and bacteria.

A *Spirochæte* not distinguishable from *S. Obermeieri* of relapsing fever has been repeatedly observed by microscopists, but I have not myself met with it.

The *Bacillus* shown in Figure 3, Plate XII, I have reason to believe, from the frequency with which I have found it, is almost

[1] Robin, Billroth, Butlin, Rappin, and the other authors referred to on page 168.

as commonly present in the healthy human mouth as is the larger and more widely known *Leptothrix*, or *Bacillus*, already described.

This minute organism, which would hardly be recognized without staining and the use of high-power objectives, is also found in normal fæces, if we can trust to the morphological resemblance which will be seen by a reference to Figures 5 and 6, Plate XIII, in which the amplification is the same (1,000 diameters).

Figure 3, Plate XII, is from a culture-experiment in which acid malt-extract (sterilized and tested in culture-oven) was inoculated with a little saliva from my own mouth.

In Figure 4, Plate XII, a fragment of an epithelial cell from the mouth of Dr. K. is shown. The nucleus of the cell is seen at the upper portion of the figure, near this some granules resembling micrococci, and on the margin of the cell a mass of rod-bacteria—probably *B. termo*. Referring again to Plate XIII, Figures 5 and 6, we see that this form also is found in normal fæces. To account for the presence of these organisms in the alimentary canal we have only to suppose that fully developed bacteria, or their unrecognized germs, can withstand the action of the digestive fluids in the stomach and the upper portion of the intestines, and that those, found in the lower bowel, are the direct descendants of those habitually present in the mouth, or of others taken into the stomach with food and drink.

Another organism which I have found quite constantly in specimens of saliva from healthy mouths, although never in any considerable abundance, is shown in Figure 5, Plate XII. This seems to be a *Sarcina*, and is, perhaps, identical with *S. ventriculi*, although it presents a somewhat different appearance as to form and grouping from this organism, as shown in a specimen from the stomach in my possession. I have frequently observed little clusters of this sarcina-like organism attached to the surface of epithelial cells in my own saliva and that of others, but to obtain it in abundance I have been obliged to resort to culture-experiments. The figure here given is from a specimen obtained by cultivation in acid malt-extract. This organism, as well as the bacillus shown in Figure 3 of the same plate, multiplies luxuriantly in this fluid when kept at a temperature of 36° C. It may be remarked, *en passant*, that acid malt-extract (a dilute solution) is not unlike the acid fluid ejected from the stomach in cases of obstinate vomiting

attended with the abundant development of *Sarcina ventriculi* in the stomach.

Figure 6, Plate XII, represents a micrococcus which possesses an especial interest because of its abundant and constant presence in the human mouth and because it has been shown to possess pathogenic properties when injected beneath the skin of a rabbit. This fact has been brought to light by recent experiments made independently by Pasteur in France,[1] and by myself in this country,[2] and since confirmed by Vulpian.[3]

The plate accompanying the paper in which I give an account of the experimental researches referred to is headed "*Micrococcus septicus*, Cohn." When this paper was written I thought it probable that the organism represented in my photo-micrographs was identical with the micrococcus described by Cohn and other observers under this name. I pointed out, however, that this micrococcus is larger than that described by Cohn as *M. septicus*, the diameter of which is given as 0.5μ, while the organism in question measures very nearly 1μ. I have since met with a smaller septic micrococcus which corresponds with Cohn's measurements, and am now inclined to believe that the micrococcus found in the human mouth is a distinct species, or at least a well established variety, differing in size but having nearly the same physiological action as the *M. septicus* of Cohn.[4]

[1] Comptes rendus Ac. d. Sc., 1881, XCII, p. 159.

[2] Bulletin National Board of Health, April 30th, 1881.

[3] Bull. de l'Acad. de Méd., March 29th, 1881.

[4] The smaller septic micrococcus above referred to was found under the following circumstances:

EXPERIMENT No. 1, Baltimore, Md., July 9th, 1881.—Injected beneath the skin of a small rabbit a little material scraped from the mucous membrane of the intestine of a rabbit just dead. (This rabbit died from an experimental injection, not yet reported, made for Professor Mallet of the University of Virginia. It presented upon post-mortem examination evidence of enteritis.) *Result:* Found dead at 8 A. M., July 10th. Diffuse cellulitis extending from point of injection; abundance of minute micrococci in serum from cellular tissue and in blood from axillary vein; liver, heart, and lungs normal; spleen enlarged and softened, but contains no pigment.

EXPERIMENT No. 2, July 10th.—A hypodermic syringe point was dipped in the blood—from femoral vein—of this rabbit and introduced under the skin of rabbit No. 2. *Result:* This rabbit was found dead the following morning at 8.30, and a post-mortem examination was made at once with the following result: Diffuse cellulitis with hemorrhagic extravasations under the skin; blood from superficial veins full of micrococci; spleen enlarged, softened, dark

In Figure 6, Plate XII, the micrococcus from the mouth is seen as obtained by cultivation (in chicken-*bouillon* inoculated with saliva) in the form of apparatus described on page 169, in which provision is made for a constantly renewed supply of the culture-fluid.

A vigorous development is shown by the grouping in long torula-chains and in zoöglœa masses. In Figure 5, Plate XI, the same organism is shown as found in a culture-flask similar to those shown in Figure 1, Plate XI. In this case the culture-fluid was inoculated with a small quantity of blood taken directly from the vessels of a rabbit just dead as the result of a sub-cutaneous injection of saliva.

The drop of blood used to inoculate the culture-fluid contained the form shown in Figure 6, Plate XI, which differs from that shown in Figure 5 and in Figure 6, of Plate XII, in having a broader aureole of transparent material. Identity is proved, however, by the fact that it is descended directly from the last form (Figure 6, Plate XII) and that the first (Figure 5, Plate XI), of which it is the progenitor, is morphologically identical with that from which it originated. A reference to Figure 3, Plate VII, in my translation of Magnin's work, "The Bacteria," will show this micrococcus upon an epithelial cell obtained directly from my own mouth. Here also I detect no morphological difference from the form obtained by cultivation in a *bouillon* made from the flesh of a chicken or of a rabbit.

The fact that this micrococcus is the most common organism found in the human mouth and that it has been described by several observers at distant points may seem difficult to reconcile

colored, has rounded edges; liver light colored; lungs congested and present numerous points of hemorrhagic infraction.

EXPERIMENT No. 3, July 11th.—A hypodermic syringe needle was dipped in blood from left auricle of rabbit No. 2 and introduced under the skin of a small rabbit (No. 3). *Result:* This rabbit died at 4.30 P. M., July 13th, but circumstances prevented me from making a careful post-mortem examination, and I have not since had an opportunity to make a more extended study of this form of septicæmia, which, so far as I am able to judge from the experiments made, differs somewhat from the form previously studied by me (*l. c.*). The spleen was not so much enlarged and was softer, with rounded edges, corresponding with the spleen of septicæmia as described by Klebs and Tommasi-Crudeli, in their mémoir upon the nature of malarial fever (Studi sulla Natura della Malaria, Roma, 1879). The inflammatory oedema or "diffuse cellulitis" was also less marked.

with the fact, recently developed, that to its presence is due the *exceptional* virulence of the saliva of certain individuals. It accords, however, with the results of recent investigations, which, as already stated in the introduction to this paper, indicate that pathogenic organisms may differ greatly as to their virulent properties as the result of different conditions relating to their environment acting upon successive generations.

My observations lead me to believe that, having a suitable medium, a proper temperature, and a sufficient supply of oxygen, the development or intensification of pathogenic properties depends to a great extent upon an abundant and constantly renewed supply of pabulum. Now this is a condition which differs greatly in the mouths of different individuals. In my own case there is, and has been from my earliest recollection, a very copious secretion of saliva. This, according to my view, accounts for the exceptional virulence which my experiments show it to possess, and is in conformity with the principles of natural selection.

Rapid multiplication is, I infer, an evidence of vigor. Now it is evident that in a natural culture-apparatus like the human mouth the rapid flow of saliva by which contained organisms are constantly washed away will have a tendency to sort out those which develop slowly from those which develop rapidly, and that the former will tend to disappear entirely, while the latter by virtue of their rapid multiplication will survive and the tendency will constantly be to a further development of this property of rapid multiplication. My culture-experiments have shown me that, in fact, this particular micrococcus does multiply with great rapidity, and that by virtue of this quality it has the precedence over *Bacterium termo*, the presence of which in any considerable number seems to be fatal to it.

This rapidity of multiplication is shown by the fact that the sub-cutaneous injection of a minute quantity of the material containing it—in the rabbit—results within 24 to 48 hours in the development of an infinite number of micrococci in the effused serum in the cellular tissue, and in the blood of the animal, where they far outnumber the normal corpuscular elements. In my culture-flasks, also, a minute drop of this blood gives rise within a few hours to the development of such a number of micrococci that the fluid contents of the flask are invaded throughout and the pabulum needed for a continued development is exhausted. I

suspect, then, that this is the simple explanation of the phenomenon in question—exceptional virulence—and I am inclined to think that the *modus operandi* of the action of these pathogenic organisms is also to be explained by the possession of this capacity for rapid multiplication.

Nature has placed, or in other words evolution has developed, in the living tissues of animals, a resisting power against the encroachments of bacterial organisms invading and surrounding them, which is sufficient for ordinary emergencies. But when the vital resistance of the tissues is reduced, on the one hand, by wasting sickness, profuse discharges, etc., or, on the other, the vital activity of the invading parasitic organism is increased, the balance of power rests with the infinitesimal but potent micrococcus. The rapid multiplication of a micro-organism introduced beneath the skin of an animal is also an advantage in its favor in the way of forestalling the restraining influence of the inflammatory process, which is a provision of nature for building up an impenetrable wall around the invader and thus circumscribing its field of operations.

Experiment has demonstrated that, by some unknown mechanism, the ordinary bacteria of putrefaction and under certain circumstances even pathogenic organisms—*e. g.* after protective inoculations with the micrococcus of chicken-cholera or the bacillus of anthrax—may be introduced directly into the circulation without the production of evil consequences, and that after a short interval microscopical examination does not reveal their presence in the blood. It is evident that here too a capacity for rapid multiplication and the introduction in the first instance of a considerable number will be circumstances favorable to the parasite and may enable it to get the start of nature's provision for getting rid of it.

NOTE.—It has occurred to me that possibly the white corpuscles may have the office of picking up and digesting bacterial organisms when by any means they find their way into the blood. The propensity exhibited by the leucocytes for picking up inorganic granules is well known, and that they may be able not only to pick up but to assimilate, and so dispose of, the bacteria which come in their way does not seem to me very improbable in view of the fact that amœbae, which resemble them so closely, feed upon bacteria and similar organisms.

Reference has already been made to Figures 5 and 6, Plate XIII, representing the common bacterial organisms found in

normal human fæces at the moment of their being discharged from the rectum. The photo-micrographs tell the story of the abundance and variety of these organisms, but the present state of knowledge does not admit of an attempt to determine their physiological rôle in the human economy. That their constant presence in the alimentary canal is a fact without import it is difficult to believe in view of their demonstrated capacity for breaking up complex organic substances external to the body in the process of their growth and functional activity.

Figure 4, Plate XIII, shows an epithelial-cell and bacteria from the orifice of the male urethra. By gently separating the lips of the urethra and applying a thin glass cover to the moist mucous membrane, good specimens are readily obtained of the organisms commonly found in this locality.

The researches of Lister and my own experiments, shortly to be detailed, indicate that the healthy human bladder is free from parasitic vegetable organisms, and it is probable that those organisms found at the extremity of the urethral canal, being *aerobic*, do not extend any considerable distance beyond the orifice.

Lister has shown that urine drawn from the healthy human bladder with proper precautions may be kept indefinitely without undergoing change, and Pasteur as long ago as 1862 (Ann. de Chemie et de Physique, 1862, p. 52. Comptes rendus Ac. de Sc., LVIII, 1864, p. 210) claimed that the alkaline fermentation of urine is due to the presence of a micro-organism — *Micrococcus ureæ*, Cohn. This organism is described by Magnin as follows:[1] "Oval cells, isolated—diameter 1.5μ (Pasteur), 1.2 to 2μ (Cohn)—or united by 2, 4, to 8 (*torula*) in a line, straight, curved, zigzag, or even in cross-form. In urine of which it transforms the urea into carbonate of ammonia (Pasteur)."

My photo-micrographs, Figures 3 and 4, Plate XI, show what I believe to be the organism in question. The group in Figure 3 answers very nearly to the measurement given, while the arrangement shown in Figure 4 corresponds with that in Cohn's drawing (Beiträge zur Biologie der Pflanzen, Band I, Heft 2, Taf. III), although the micrococcus in this figure is smaller. It is possible that we have here two different organisms, but I am inclined to believe that the difference in size is due simply to the fact that

[1] The Bacteria. Little, Brown & Co., Boston, 1880.

different stages of development are represented, Figure 4 showing an active pullulating stage and Figure 3 a grouping of the micrococcus in masses after the completion of the transformation of the urea. A difference in the size of individual micrococci will be noticed in Figure 4, and it must be admitted that in photographing these minute organisms with high powers a very slight difference in focal adjustment makes a difference in the apparent size of the organism. Too much stress should not, therefore, be placed upon slight differences of measurement as reported by different observers and obtained by different methods.

I call attention to the fact that this micrococcus has a well defined outline and does not present the appearance of being surrounded by an aureole such as is seen in Figures 5 and 6 of the same plate. This is an additional proof that this aureole is not the result of diffraction, but that it represents a transparent substance enveloping the micrococcus. (See remarks on page 18 of Special Report on "A Fatal Form of Septicæmia," etc. Reprinted from National Board of Health Bulletin, *l. c.*)

The following experiments are reported here as relating to the rôle of this micrococcus, which, notwithstanding the researches of Pasteur, Lister, and others, is not perhaps generally admitted by chemists and physiologists to be *un fait établi.*

Having repeatedly demonstrated the presence of micrococci at the mouth of the male urethra and knowing that Lister's experiments indicate that urine as contained in the healthy bladder is free from bacterial contamination, it occurred to me that in passing urine from a full bladder the first portion of the stream might wash away detached epithelial-cells and bacterial organisms, and that the last portion being received in a sterilized flask might give evidence of freedom from these organisms by remaining unchanged. Accordingly I made the following

EXPERIMENT, Baltimore, Md., June 25th, 1881.—Two bell-shaped glass cups were sterilized in the flame of a Bunsen burner and placed under clean bell-jars in the position shown in Figure 2, Plate XI (Lister's Apparatus). I then desired my assistant to pass a small quantity of urine into No. 1 from the first portion of the flow and into No. 2 from the last, removing and replacing the bell-jars as expeditiously as possible. *Result*, June 30th: No. 1 is turbid, has a considerable sedimentary deposit and is decidedly alkaline. No. 2 remains perfectly transparent, has no sedimentary

deposit and is acid. No. 1 contains an abundance of the micrococci shown in Figures 3 and 4, and No. 2 is free from organisms. A single drop taken up from the bottom of No. 1 by means of a pipette was allowed to fall in No. 2. The following day No. 2 was turbid, had an alkaline reaction and contained an abundance of *Micrococcus ureæ*.

This experiment can not be expected to succeed in every instance, as the complete washing away of organisms by the first portion of the stream may not always occur, and it is possible that the previous passage of urine may have washed out the urethra so that the first urine passed will be free from organisms, while the last might be contaminated by the detachment of epithelium in which micrococci were imbedded, as seen in my photographs.

Some such explanation is necessary to account for the result obtained in the following experiment.

EXPERIMENT No. 2, Baltimore, Md., August 1st, 1881.—The above experiment was repeated with the same precautions. *Result*, August 11th: No. 1 remains acid and transparent in the upper portion, but the lower third is occupied by a loose finely granular precipitate, such as is often seen in fresh urine immediately after cooling. No. 2 has an alkaline reaction, a film of urate of ammonia upon the surface and an abundance of *Micrococcus ureæ* in the copious deposit at the bottom of the cup.

EXPERIMENT No. 3, Baltimore, Md., August 1st, 1881.—Four sterilized cups were prepared as in the preceding experiments and into each was passed a small quantity of urine from my own bladder, after first taking the precaution of disinfecting the extremity of the urethra. This was accomplished by the liberal use of a 3 per cent. solution of carbolic acid, which was applied by means of a pledget of asbestos held by slender forceps. The asbestos was first sterilized by heat and then being dipped in the disinfecting solution was repeatedly and thoroughly applied to the mucous membrane to the depth of half an inch or a little more. This operation produced some pain, and a little soreness upon passing urine was felt for two or three days. *Result:* Five days after (August 6th) the urine in all of the vessels remained transparent. At this date No. 3 was inoculated with organisms from the mouth of the urethra. This was done by twisting around in the orifice of the urethra a small ball of asbestos, previously sterilized by heat, which was then dropped into the cup containing

urine, the bell-jar being removed for an instant only for this purpose. Five days later the contents of the four cups were carefully examined. Nos. 1, 2 and 4 remained transparent, free from sedimentary deposit, and acid. *No. 3 was alkaline and contained an abundance of Micrococcus ureæ.*

A reference to Figure 4, Plate XIII, will show that the organism there seen in considerable abundance is not identical in appearance with *Micrococcus ureæ* as seen in Figures 3 and 4, Plate XI. Direct examination has not given me as satisfactory evidence of the presence of this micrococcus at the extremity of the urethra as have the experiments above detailed. It may be, however, that under different circumstances this organism assumes a different appearance, and that the form shown in Figure 4, Plate XIII, from the surface of a mucous membrane exposed to the air, when submerged in a liquid having the composition of urine undergoes a transformation into the form seen in Figures 3 and 4, Plate XI. This is a question to be settled by carefully conducted culture-experiments.

Figures 1 and 3, Plate XIII, are from the vagina of a healthy female at the termination of the menstrual flow. I shall not here dwell upon the possible import of the presence of micrococci in such numbers in this situation, but from what has already been said it seems evident that gynecologists may well be on their guard to prevent the invasion of wounds in this locality—accidental or made by the surgeon—by these ever-present parasitic organisms, and especially against the development of virulent varieties as the result of profuse and long continued discharges—puerperal, etc.

Figure 7, Plate XI, is introduced for comparison with the other micrococci upon the same plate. The amplification is the same in each—1,000 diameters. This figure is from a specimen obtained by cultivation of micrococci found in gonorrhœal pus, in chicken-*bouillon*. The reader is cautioned against the inference that this micrococcus is the cause of the virulence of the fluid in which it was found. No great weight can be attached to the mere presence of an organism under such circumstances in the absence of culture- and inoculation-experiments to demonstrate its physiological properties. Such experiments I have had no opportunity of making in this case, and the figure is introduced solely for the purpose of showing that distinct morphological differences may be recognized between these micrococci from three different sources, viz: from the human mouth, from urine, and from gonorrhœal pus.

DESCRIPTION OF PLATES.

PLATE XI.

FIGURE 1.—Culture-flasks in position for introduction into culture-oven.

FIGURE 2.—Lister's Apparatus (slightly modified).

FIGURE 3.—*Micrococcus ureæ*, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. hom. ol. im. objective; aniline brown staining.

FIGURE 4.—Same as Figure 3 (see remarks on p. 171).

FIGURE 5.—Micrococcus cultivated in *bouillon* (rabbit flesh) inoculated with blood from septicæmic rabbit, and descended from common micrococcus found in the healthy human mouth, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—The same micrococcus as in Figure 5, as it appears in the blood of rabbit killed by the sub-cutaneous injection of human saliva, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective. Iodine staining.

FIGURE 7.—Micrococcus from culture-experiment with gonorrhœal pus × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

PLATE XII.

FIGURE 1.—*Leptothrix buccalis*, obtained directly from mouth, × 1,000 by Zeiss's $\frac{1}{12}$ in. objective.

FIGURE 2.—*Leptothrix buccalis* from culture-experiment, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 3.—*Bacillus* (sp. ?) from culture-experiment (saliva in malt-extract), × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 4.—Portion of epithelial-cell from mouth (Dr. K.) covered with bacteria (*B. termo?*), × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 5.—*Sarcina* (ventriculi ?) from saliva-culture in acid malt-extract, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—*Micrococcus* from saliva-culture in chicken-*bouillon*, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

PLATE XIII.

FIGURE 1.—Epithelium and micrococci from vagina, × 425 diameters by Beck's $\frac{1}{6}$ in. objective.

FIGURE 2.—Epithelium and *Leptothrix buccalis* from mouth, × about 300 diameters by Beck's $\frac{1}{6}$ in. objective.

FIGURE 3.—Epithelial-cell and micrococci from vagina, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 4.—Epithelial-cell and bacteria from extremity of male urethra, × 1,000 by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 5.—Bacterial organisms in normal and recently discharged human fæces, × 1,000 diameters by Zeiss's $\frac{1}{18}$ in. objective.

FIGURE 6.—The same as Figure 5.

Fig. 1.

Fig. 5.

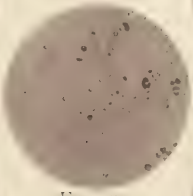


Fig. 7.

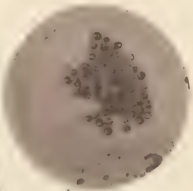
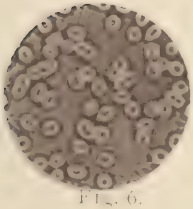


Fig. 3.



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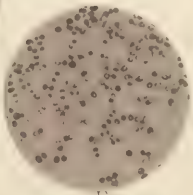

Fig. 4.

Fig. 2.

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Fig. 2

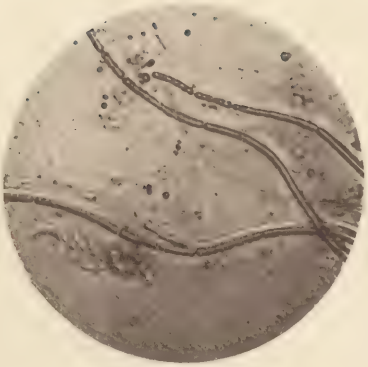
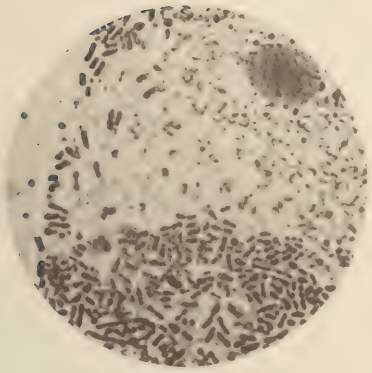


Fig. 1.



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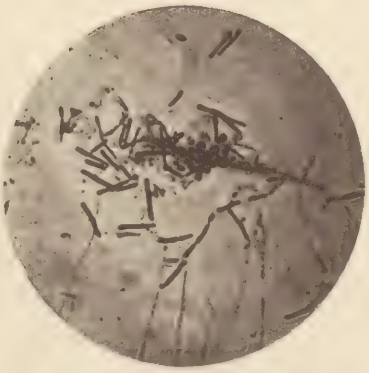

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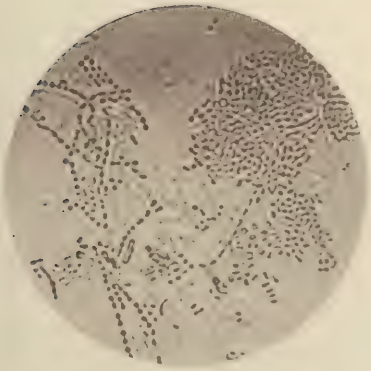

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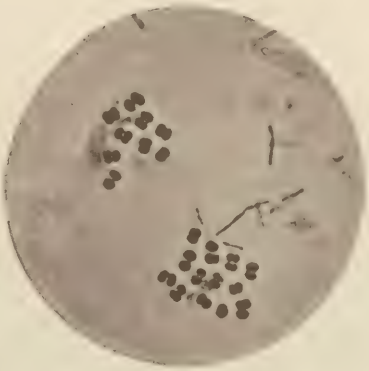

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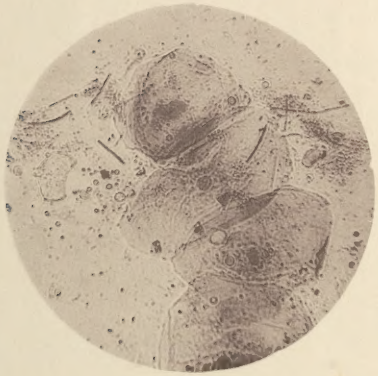


Fig. 2.

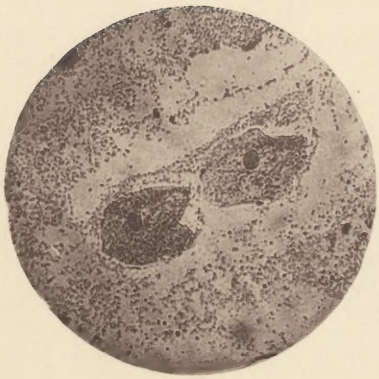
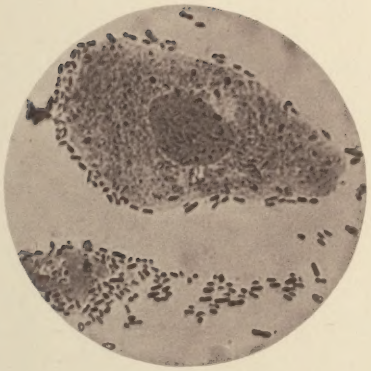


Fig. 1.



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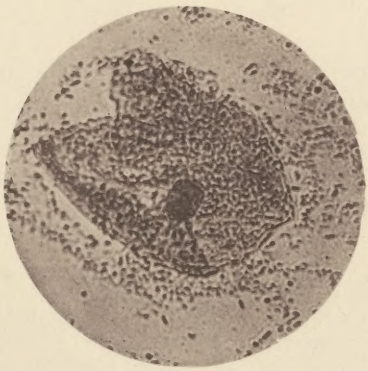

Fig. 3.

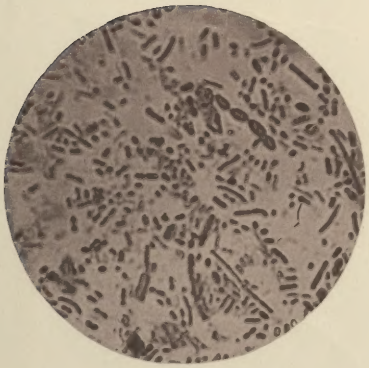

Fig. 6.

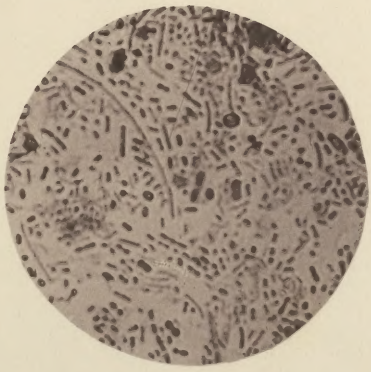

Fig. 5.

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